

Pharmacochemistry

Steroidal Glycosides from the Leaves of *Yucca Gloriosa* L.

Ether Kemertelidze*, Mariam Benidze**, Alexandr Skhirtladze**

* Academy Member, I.Kutateladze Institute of Pharmacochemistry, Tbilisi

** I.Kutateladze Institute of Pharmacochemistry, Tbilisi

ABSTRACT. The existence of 24 steroidal glycosides, including 13 furostanols was confirmed in air-dried leaves of plant *Yucca gloriosa* introduced in Georgia. Most of glycosides are derivatives of tigogenin. 14 glycosides, among them 4 new organic substances, have been isolated and characterized, using classical and modern methods of spectral analysis. © 2011 Bull. Georg. Natl. Acad. Sci.

Key words: steroidal glycosides, spirostanol, furostanol.

Previously it was shown at the Institute of Pharmacochemistry of the Georgian National Academy of Sciences that the leaves of *Yucca gloriosa* L. (YG) grown in Georgian botanical gardens generally biosynthesize steroidal sapogenin-tigogenin which, accordingly, simplifies its isolation from the raw.

Tigogenin from YG has been transformed into initial products for the synthesis of steroidal hormonal drugs: acetate 5 α -androstan-3 β -ol-17-on and acetate-5 α -pregn-16-en-3 β -20-on, and later the synthesis of a number of hormonal preparations was carried out [1-3]. Tigogenin was recognized by the Ministry of Medical Industry of the former Soviet Union as cost-effective industrial raw material for the synthesis of 5 α steroidal line hormonal drugs. 190 ha plantations of YG have been set up in Eastern Georgia in accordance with agrarian recommendations elaborated at the Institute to satisfy the need in vegetable raw [4].

YG is a perennial evergreen, frost- and drought-resistant, plentifully blossoming wonderful decorative shrub. It is widely involved in landscape gardening of Tbilisi and other regions of Georgia.

All parts of YG are rich in steroidal compounds. Lots of steroidal glycosides including new organic compounds have been isolated from the leaves, flowers, roots, rhizomes and characterized [5-8]. 11 new compounds and interesting spirostructure stilbens, exhibitig high antioxi-

dant, pro-apoptotic and antiproliferative activity were established in roots, rhizomes and stem bark [9-12]. The presumptive fungicide preparation "Gloriofucin" was developed on the basis of spirostanolic glycosides from YG leaves, dried up on the bottom circles of a live plant [13]. Crude steroidal glycosides from YG flowers represent the active substance of agricultural crops growth stimulator – "Alexin" [14].

Furostanolic steroidal glycosides are localized in mesophyllic cells of YG leaves, whereas the hydrolyzed enzyme- β -glucosidase-in the epidermis. The drying off process breaks the integrity of cells, and the enzyme reacts with furostanol removing its side-chain carbohydrate: as a result the open core closes transforming the glycoside into spiro-form [15]. According to the features of enzymatic process different amounts of furo- and spirostans may be found.

This work touches on the study of steroidal glycosides from air-dried leaves of YG. The raw was harvested from the experimental field of medicinal plants of the Institute of Pharmacochemistry in May 2008.

100 g of air-dried leaves was once extracted with 400 ml of absolute methanol at room temperature and then twice in 300-300 ml 80% of methanol at its boiling temperature. After the evaporating of methanol from the pool extract the remaining aqueous solution was threefold treated with 50-50 ml of chloroform in order to remove

lipophilic substances, and then the steroidal glycosides were extracted with fivefold 70-70 ml n-butanol treatment. Butanol was evaporated, and the residue was dried and ground. 5.5 g of amorphous yellowish white powder-crude saponins were obtained. Then crude saponins were subjected to HPLC/MS/MS analysis on Thermo Finnigan LCQ Deca ion trap mass-spectrometer (Thermo Finnigan, San Jose, CA, USA). HPLC partitioning was carried out on the reversed-phase column (5 mm, 2.1x250; X-Terra MS C18; Waters, Milford, MA), mobile phase: H₂O (A) and CH₃CN (B) at 0.2 ml/min, gradient conditions: 0 min-5% B, 55 min-60% B, 60 min-90% B.

HPLC/MS/MS analysis of air-dried extract of YG leaves confirmed the presence of 24 steroidal glycosides.

As shown in the table, after the MSⁿ of glycosides **1-10**, **17**, **19** and **24** the water and the corresponding hexose fragments (m/z 18 and m/z 162) were removed. Such fragmentation is characteristic of furostans due to the presence of free hydroxyl at C-22 and glucose at C-26. All glycosides, except for **10**, **23** and **24**, have a side-chain containing 4-7 monosaccharides, attached to C-3, which is proved by the removing of two terminal sugars from each of them.

Separation of 5 g of air-dried leaves butanolic extract

Table 1

HPLC/ESIMS and ESIMS/MS fragmented ions of YG leaves air-dried extract

	molecular ions*	retention time, min	MS ⁿ fragmented ions
1	1715 [M+H] ⁺ , F	22.49	1535 [M-18-162+H] ⁺ ; 1389 [M-18-162-146+H] ⁺ ; 1373 [M-18-162-162+H] ⁺ ; 741 [M-18-162-146-162-162+H] ⁺
2	1407 [M+H] ⁺ , F	22.59	1227 [M-18-162+H] ⁺ ; 1081 [M-18-162-146+H] ⁺ ; 1065 [M-18-162-162+H] ⁺ ; 595 [M-18-162-146-162-162+H] ⁺ ; 433 [M-18-162-146-162-162-162+H] ⁺
3	1377 [M+H] ⁺ , F	22.79	1197 [M-18-162+H] ⁺ ; 1051 [M-18-162-146+H] ⁺ ; 1035 [M-18-162-162+H] ⁺ ; 595 [M-18-162-146-162-132-162+H] ⁺
4	1525 [M+H] ⁺ , F	26.68	1345 [M-18-162+H] ⁺ ; 1213 [M-18-162-132+H] ⁺ ; 1183 [M-18-162-162+H] ⁺ ; 1051 [M-18-162-132-162+H] ⁺ ; 919 [M-18-162-132-162-132+H] ⁺ ; 433 [M-18-162-132-162-132-162-162+H] ⁺
5	1553 [M+H] ⁺ , F	30.36	1373 [M-18-162+H] ⁺ ; 1227 [M-18-162-146+H] ⁺ ; 1211 [M-18-162-162+H] ⁺ ; 579 [M-18-162-146-162-162-162+H] ⁺
6	1509 [M+H] ⁺ , F	32.20	1329 [M-18-162+H] ⁺ ; 1197 [M-18-162-132+H] ⁺ ; 1167 [M-18-162-162+H] ⁺ ; 903 [M-18-162-132-162-132+H] ⁺
7	1523 [M+H] ⁺ , F	33.10	1343 [M-18-162+H] ⁺ ; 1197 [M-18-162-146+H] ⁺ ; 1181 [M-18-162-162+H] ⁺ ; 903 [M-18-162-146-162-162+H] ⁺
8	1245 [M+H] ⁺ , F	34.49	1065 [M-18-162+H] ⁺ ; 903 [M-18-162-162+H] ⁺ ; 741 [M-18-162-162-162+H] ⁺
9	1215 [M+H] ⁺ , F	34.92	1035 [M-18-162+H] ⁺ ; 903 [M-18-162-132+H] ⁺ ; 741 [M-18-162-132-162+H] ⁺
10	921 [M+H] ⁺ , F	35.74	741 [M-18-162+H] ⁺ ; 417 [M-18-162-162-162+H] ⁺
11	1197 [M+H] ⁺ , S	36.30	1051 [M-146+H] ⁺ ; 1035 [M-162+H] ⁺ ; 595 [M-146-162-132-162+H] ⁺
12	1065 [M+H] ⁺ , S	36.77	741 [M-162-162+H] ⁺
13	1227 [M+H] ⁺ , S	37.03	1081 [M-146+H] ⁺ ; 1065 [M-162+H] ⁺ ; 433 [M-146-162-162-162+H] ⁺ ; 1213 [M-132+H] ⁺ ; 1183 [M-162+H] ⁺ ; 1051 [M-18-162-132-162+H] ⁺ ; 433 [M-132-162-132-162-162-162+H] ⁺
14	1345 [M+H] ⁺ , S	37.29	741 [M-132-162+H] ⁺
15	1035 [M+H] ⁺ , S	37.39	1197 [M-132+H] ⁺ ; 1167 [M-162+H] ⁺ ; 903 [M-132-162-132+H] ⁺
16	1329 [M+H] ⁺ , S	37.54	1211 [M-18-162+H] ⁺ ; 1065 [M-18-162-146+H] ⁺ ; 1049 [M-18-162-146-162+H] ⁺ ; 741 [M-18-162-146-162-162+H] ⁺
17	1391 [M+H] ⁺ , F	37.63	1227 [M-146+H] ⁺ ; 1211 [M-162+H] ⁺ ; 579 [M-146-162-162-162-162+H] ⁺
18	1373 [M+H] ⁺ , S	37.95	1181 [M-18-162+H] ⁺ ; 1035 [M-18-162-146+H] ⁺ ; 989 [M-18-162-162+H] ⁺ ; 741 [M-18-162-146-162-132+H] ⁺ ; 579 [M-18-162-146-162-132-162+H] ⁺
19	1361 [M+H] ⁺ , F	38.36	1197 [M-146+H] ⁺ ; 1181 [M-162+H] ⁺ ; 903 [M-146-162-162+H] ⁺ ; 1065 [M-146+H] ⁺ ; 1049 [M-146-162+H] ⁺ ; 579 [M-146-162-162-162+H] ⁺ ; 1035 [M-146+H] ⁺ ; 989 [M-162+H] ⁺ ; 741 [M-146-162-132+H] ⁺
20	1343 [M+H] ⁺ , S	38.70	417 [M-162-162+H] ⁺
21	1211 [M+H] ⁺ , S	39.16	579 [M+H-18] ⁺ ; 417 [M-18-162+H] ⁺
22	1181 [M+H] ⁺ , S	39.58	
23	741 [M+H] ⁺ , S	40.25	
24	597 [M+H] ⁺ , F	41.52	

* F: furostanol; S: spirostanol

on silicagel column (L100/160) using mobile phase chloroform-methanol-water 26:14:3 resulted in obtaining glycosides **11** (19 mg), **15** (10 mg), **18** (27 mg), **20** (7 mg), **21** (32 mg), **22** (8 mg), **23** (20 mg), and enriched fractions A (152 mg), B (203 mg) and C (185 mg). Follow-up HPLC separation of A, B and C on reverse phase column (7.8 x 300 mm, LiChroprep RP18, 10 mm, XTerra) with different concentrations of methanol in isocratic conditions at a rate 2ml/min allowed to obtain 7 individual furostanolic glycosides: **10** (8 mg) and **24** (17 mg) from **A** (65% MeOH); **9** (6 mg), **17** (12 mg) and **19** (5 mg) from **B** (60% MeOH); **1** (14 mg) and **5** (9 mg) fraction from **C** (57% MeOH).

The structures of isolated compounds were established by one- (^1H , ^{13}C , 1D-TOCSY) and two-dimensional (HSQC, HMBC, COSY) experiments.

10 compounds appeared to be known glycosides with the following structures:

(25R)-26-0- β -D-glucopyranosyl-5 α -furostan-3 β ,26-diol 3-0- β -D-xylopyranosyl-(1 \rightarrow 3)-[0- β -D-glucopyranosyl-(1 \rightarrow 2)]-0- β -D-galactopyranoside (**9**); (25R)-26-0- β -D-glucopyranosyl-5 α -furostan-3 β , 26-diol 3-0- β -D-glucopyranosyl-(1 \rightarrow 2)-0- β -D-galactopyranoside (**10**); (25R)-26-0- β -D-glucopyranosyl-5 α -furostan-3 β , 26-diol 3-0- α -L-rhamnopyranosyl-(1 \rightarrow 4)-0- β -D-glucopyranosyl-(1 \rightarrow 3)-[0- β -D-glucopyranosyl-(1 \rightarrow 2)]-0- β -D-glucopyranosyl-(1 \rightarrow 4)-0- β -D-galactopyranoside (**17**); (25R)-5 α -spirostan-3 β -ol 3-0- β -D-glucopyranosyl-(1 \rightarrow 2)-0- β -D-galactopyranoside (**23**); (25R)-5 α -spirostan-3 β -ol 3-0- β -D-xylopyranosyl-(1 \rightarrow 3)-[0- β -D-glucopyranosyl-(1 \rightarrow 2)]-0- β -D-glucopyranosyl-(1 \rightarrow 4)-0- β -D-galactopyranoside (**15**); (25R)-5 α -spirostan-3 β ,2 α -diol 3-0- α -L-rhamnopyranosyl-(1 \rightarrow 4)-0- β -D-xylopyranosyl-(1 \rightarrow 3)-[0- β -D-glucopyranosyl-(1 \rightarrow 2)]-0- β -D-glucopyranosyl-(1 \rightarrow 4)-0- β -D-galactopyranoside (**11**); (25R)-5 α -spirostan-3 β -ol 3-0- α -L-rhamnopyranosyl-(14)-0- β -D-xylopyranosyl-(1 \rightarrow 3)-[0- β -D-glucopyranosyl-(1 \rightarrow 2)]-0- β -D-glucopyranosyl-(1 \rightarrow 4)-0- β -D-galactopyranoside (**22**); (25R)-5 α -spirostan-3 β -ol 3-0- α -L-rhamnopyranosyl-(1 \rightarrow 4)-0- β -D-xylopyranosyl-(1 \rightarrow 3)-[0- β -D-glucopyranosyl-(1 \rightarrow 3)]-0- β -D-xylopyranosyl-(1 \rightarrow 2)]-0- β -D-glucopyranosyl-(1 \rightarrow 4)-0- β -D-galactopyranoside (**21**); (25R)-5 α -spirostan-3 β -ol 3-0- α -L-rhamnopyranosyl-(1 \rightarrow 4)-0- β -D-xylopyranosyl-(1 \rightarrow 3)-[0- β -D-glucopyranosyl-(1 \rightarrow 3)]-0- β -D-glucopyranosyl-(1 \rightarrow 2)]-0- β -D-glucopyranosyl-(1 \rightarrow 4)-0- β -D-galactopyranoside (**20**); (25R)-5 α -spirostan-3 β -ol 3-0- α -L-rhamnopyranosyl-(1 \rightarrow 4)-0- β -D-glucopyranosyl-(1 \rightarrow 3)-[0- β -D-xylopyranosyl-(1 \rightarrow 3)]-0- β -D-glucopyranosyl-(1 \rightarrow 2)]-0- β -D-glucopyranosyl-(1 \rightarrow 4)-0- β -D-galactopyranoside (**18**) [5-8, 14].

Compounds identical to glycosides **1**, **5**, **9**, **24** were

not described in the literature so far, and turned out to be novel ones; therefore appropriate experimental work was carried out for their characterization.

Furostanol 1 - white amorphous powder, gives a positive reaction with Ehrlich's reagent. **Acid hydrolysis**: 10 mg of the substance is heated at 95°C with 8 ml 1.0 N HCl for 3 hours. After the cooling the laid-down residue was filtered, washed with distilled water until negative reaction and dried, yielding 2 mg of aglycone - white crystalline powder with melting temperature 201-204°C. IR spectrum reveals the absorbance maximum λ_{max} (KBr): 3300 (OH), 1030, 993, 958, 921, 895, 866 cm^{-1} ; 921 < 895 (25R). TLC in solvent system CHCl_3 -MeOH 10:1 gives one spot at a level of tigogenin standard. Mixing the sample with tigogenin does not cause depression of melting point and appears on TLC as one inseparable spot. Thus, the aglycone of furostanol **1** turned out to be tigogenin. Filtrate was neutralized on KY-2 ion-exchange column, dried up, solved in 1-2 drops of methanol-water mixture. Sugar content was analyzed by paper chromatography vs standard samples under the following conditions: mobile phase - pyridine-benzol-butanol-water 5:1:5:3; developer - aniline phthalate, t-100°C. Sugar spots were detected at D-glucose, L-rhamnose, and D-galactose level.

MS of furostanol **1** shows a positive ion peak m/z 1715 $[\text{M}+\text{H}]^+$ and fragments m/z 1697 $[\text{M}+\text{H}-18]^+$, m/z 1535 $[\text{M}+\text{H}-18-162]^+$, m/z 1389 $[\text{M}+\text{H}-18-162-146]^+$, m/z 1227 $[\text{M}+\text{H}-18-162x2-146]^+$, m/z 417 $[\text{M}+\text{H}-18-162x7-146]^+$, and corresponds to molecular formula $\text{C}_{75}\text{H}_{126}\text{O}_{43}$. ^1H NMR spectrum shows two singlets of tertiary methyl δ 0.82 (Me-18, s), δ 0.86 (Me-19, s), two doublets of secondary methyl δ 0.94 (Me-21, d), δ 1.00 (Me-27, d), two secondary δ 3.68 (H-3, m), δ 4.56 (H-16), two primary alcohol function signals δ 3.39 (H-26a, dd), δ 3.75 (H-26b, dd), and eight anomeric signals δ 4.51 (H-1, GlcI), δ 5.03 (H-1, GlcII), δ 4.93 (H-1, GlcIII), δ 4.85 (H-1, Rha), δ 4.71 (H-1, GlcIV), δ 4.60 (H-1, GlcV), δ 4.38 (H-1, Gal), δ 4.25 (H-1, GlcVI). The essence of monosaccharides was established by 1D-TOCSY experiment (Table 3). The fact that aglycone belongs to 5 α line was proved by the presence of δ 38.1, 45.7, 55.5 chemical shifts corresponding to C-1, C-5 and C-9 carbon atoms in ^{13}C spectrum. Methyl group at C-25 is of R configuration, because the difference in pair of protons H2-26 equals 0.36 [7]. The ^1H and ^{13}C spectral data (Table 2) also confirm that the aglycone is tigogenin. Substitution of glucose in 26th position was found from HMBC correlation between glucose anomeric proton δ 4.25 (H-1, GlcVI) and aglycone carbon C-26 (δ 75.8). HMBC spectrum shows significant correlation peaks between sugar anomeric signals and glycolized carbons: δ 4.51 (H-1, GlcI) \rightarrow δ 87.2 (C-3, GlcII), δ 5.03 (H-1, GlcII) \rightarrow δ 80.4

(C-4, GlcIII), δ 4.93 (H-1, GlcIII) \rightarrow δ 80.6 (C-2, GlcV), δ 4.85 (H-1, Rha) \rightarrow δ 78.9 (C-4, GlcIV), δ 4.71 (H-1, GlcIV) \rightarrow δ 87.6 (C-3, GlcV), δ 4.60 (H-1, GlcV) \rightarrow δ 79.7 (C-4, Gal), δ 4.38 (H-1, Gal) \rightarrow δ 78.9 (C-3, Agl), δ 4.25 (H-1, GlcVI) \rightarrow δ 75.8 (C-26, Agl).

On the basis of the obtained data furostanol **1** was characterized as: (25R)-26- β -D-glucopyranosyl-5 α -furostan-3 β , 26-diol 3- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 3)-[0- β -D-glucopyranosyl-(1 \rightarrow 3)-0- β -D-glucopyranosyl-(1 \rightarrow 4)-0- β -D-glucopyranosyl-(1 \rightarrow 2)]-0- β -D-glucopyranosyl-(1 \rightarrow 4)-0- β -D-galactopyranoside.

Structure determination of novel furostanols was realized using one and the same scheme. Here we present the spectral data only of glycoside with the most complex carbohydrate residue – aglycone of furostanol **1**.

Furostanol 5 - white amorphous powder, gives a positive reaction with Ehrlich's reagent. Acid hydrolysis of the compound (6 mg) was carried out as described above.

Tigogenin (1.2 mg) and monosaccharides D-glucose, L-rhamnose, and D-galactose were obtained.

MS shows a positive ion peak m/z 1553 $[M+H]^+$ and fragments m/z 1373 $[M-18-162+H]^+$; 1227 $[M-18-162-146+H]^+$; 1211 $[M-18-162x2+H]^+$; 579 $[M-18-162x5-146+H]^+$ corresponding to molecular formula $C_{69}H_{116}O_{38}$. In aglycone there are 5 oxy- and 1 desoxy sugars substituted at C-3, with anomeric signals δ 4.78 (H-1 Rha), 5.05 (H-1 GlcI), 4.92 (H-1 GlcII), 4.70 (H-1 GlcIII), 4.62 (H-1 GlcIV), 4.39 (H-1 Gal). Sugar chain joining sequence in aglycone is similar to furostanol **1** except for one glucose.

Therefore, the structure of furostanol **5** can be interpreted as: (25R)-26- α -D-glucopyranosyl-5 α -furostan-3 β , 26-diol 3- α -L-rhamnopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl-(1 \rightarrow 3)-[0- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 2)]-0- β -D-glucopyranosyl-(1 \rightarrow 4)-0- β -D-galactopyranoside.

Furostanol 19 - white amorphous powder, gives a positive reaction with Ehrlich's reagent. Acid hydrolysis

Table 2

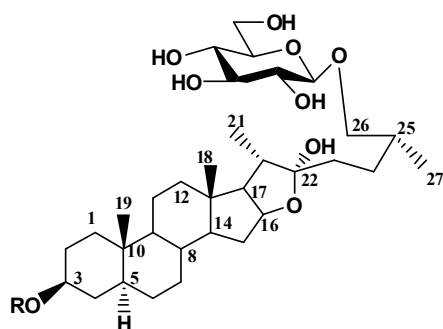
 ^{13}C and 1H NMR chemical shifts of tigogenin – aglycone of furostanol **1**

№	furostanol 1		№	furostanol 1	
	^{13}C	1H		^{13}C	1H
1	38.1	1.74-1.00	15	32.7	1.96-1.23
2	30.2	1.87-1.54	16	81.9	4.56
3	78.9	3.68	17	63.7	1.78
4	34.6	1.70-1.33	18	16.2	0.82
5	45.7	1.11	19	12.3	0.86
6	29.4	1.32	20	40.2	2.11
7	32.9	1.72-0.95	21	16.5	0.94
8	36.0	1.60	22	111.8	-
9	55.5	0.69	23	32.6	1.80-1.41
10	36.3	-	24	28.6	1.67-1.20
11	21.4	1.54-1.35	25	37.3	1.74
12	40.5	1.76-1.16	26	75.8	3.75-3.39
13	41.7	-	27	15.6	1.00
14	57.2	1.15			

Table 3

 ^{13}C and 1H NMR chemical shifts of sugars of furostanol **1**

Rha	GlcI	GlcII	GlcIII	GlcIV	GlcV
4.85-102.2	4.51-105.6	5.03-103.3	4.93-103.7	4.71-103.6	4.60-104.1
3.84-71.9	3.26-77.4	3.39-74.6	3.19-74.9	3.30-74.8	3.79-80.6
3.63-75.0	3.37-77.7	3.53-87.2	3.37-71.1	3.48-76.4	3.78-87.6
3.41-73.2	3.29-75.6	3.43-69.3	3.78-80.4	3.55-78.9	3.44-77.7
3.98-70.2	3.30-74.8	3.37-77.7	3.35-70.3	3.53-70.4	3.36-74.3
1.27-17.5	3.92-62.2	3.91-62.2	3.91-62.2	3.93-60.8	3.88-62.3
	3.78	3.82	3.83	3.63	3.67
Gal	Glc \rightarrow C-26				
4.38-102.0	4.25-104.0				
3.62-72.7	3.19-74.9				
3.53-75.0	3.37-77.6				
4.04-79.7	3.28-71.1				
3.64-71.7	3.29-77.5				
3.91-62.2	3.91-62.4				
3.82	3.72				

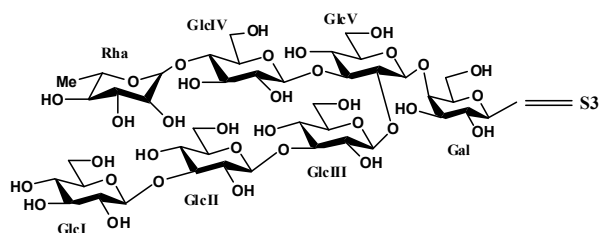
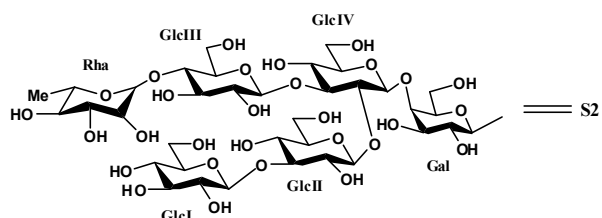
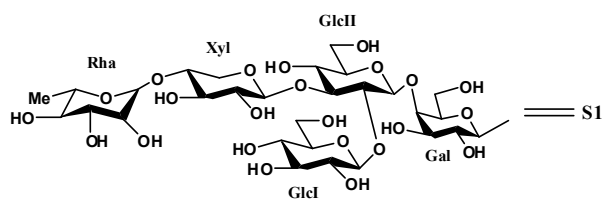


1. R=S3; 5. R=S2; 19. R=S1; 24. R=H.

of the compound (4 mg) was carried out as of furostanol **1**. Tigogenin (1.0 mg) and monosaccharides *D*-glucose, *D*-xylose, *D*-galactose and *L*-rhamnose were obtained.

MS shows a positive ion peak m/z 1361 $[M+H]^+$ and fragments m/z 1181 $[M-18-162+H]^+$; 579 $[M-18-162 \times 3-146-132+H]^+$, corresponding to the molecular formula $C_{62}H_{104}O_{32}$. Aglycone of furostanol **19** is tigogenin. The difference is only in features, amount and substitution order of sugar at C-3. 1H NMR spectrum shows anomeric signals for 6 sugars: δ 4.72 (H-1 Rha), 4.50 (H-1 Xyl), 4.90 (H-1 GlcI), 4.62 (H-1 GlcII), 4.39 (H-1 Gal) and 4.26 (H-1 GlcIII). HMBC spectrum reveals significant correlation peaks between anomeric signals of sugar and glycolized carbon: δ 4.90 (H-1, GlcI) \rightarrow δ 80.4 (C-2, GlcII), δ 4.72 (H-1, Rha) \rightarrow δ 75.9 (C-4, Xyl), δ 4.50 (H-1, Xyl) \rightarrow δ 87.8 (C-3, GlcII), δ 4.62 (H-1, GlcII) \rightarrow δ 79.6 (C-4, Gal), δ 4.39 (H-1, Gal) \rightarrow δ 78.7 (C-3, Agl), δ 4.26 (H-1, GlcIII) \rightarrow δ 75.6 (C-26, Agl). Therefore furostanol **19** was characterized as (25*R*)-26-*O*- β -*D*-glucopyranosyl-5 α -furostan-3 β , 26-diol 3-*O*- α -*L*-rhamnopyranosyl-(1 \rightarrow 4)-*O*- β -*D*-xylopyranosyl-(1 \rightarrow 3)-[*O*- β -*D*-glucopyranosyl-(1 \rightarrow 2)]-*O*- β -*D*-glucopyranosyl-(1 \rightarrow 4)-*O*- β -*D*-galactopyranoside.

Furostanol 24 – white amorphous powder, gives a positive reaction with Ehrlich's reagent. Acid hydrolysis of 10 mg of the substance gives 7 mg tigogenin and *D*-glucose. MS shows a positive ion peak m/z 597 $[M+H]^+$ and fragments m/z 579 $[M+H-18]^+$, m/z 417 $[M+H-18-162]^+$, molecular formula $C_{33}H_{56}O_9$, 1H NMR spectrum shows two singlets of tertiary methyls δ 0.84 (Me-18, s), δ 0.88 (Me-19, s), two doublets of secondary methyls δ 0.98 (Me-21, d), δ 1.02 (Me-27, d), two secondary δ 3.53 (H-3, m), δ 4.38



(H-16) and two primary alcohols function δ 3.41 (H-26a, dd), δ 3.77 (H-26b, dd), and the anomeric signal of glucose δ 4.27 (H-1 Glc, d). Glucose substitution in C-26 position was established by HMBC correlation between glucose anomeric proton and aglycone C-26 carbon atoms (d 75.8).

Thus, the structure provided for furostanol **24** is: (25*R*)-5 α -furostan-3 β , 26-diol 26-*O*- β -*D*-glucopyranoside.

Thus, an interesting composition of steroids is established from leaves of YG, cultivated in Georgia. Among 24 glycosides found 13 are furostanols, 11 – spirostanols. Most of them are derivatives of tigogenin; six (2,3,4,11,13,14) of 2 α ,3 β diol-5 α -steroid-derivatives of tigogenin, and two (10,23) 5 β , 3 β , - derivatives of sapogenin smilagenin. The carbohydrate part of the obtained compounds consists of glucose (dominating sugar), galactose, rhamnose and xylose. The significant amount of polar glycosides should be mentioned. Among substances found in YG leaves there are 2 biosides, 4 tetraosides, 10 pentaosides, 6 hexaosides and 1 heptaoside. Complex composition of the glycosides of YG leaves presumes their interesting pharmacological activity and creates important prospects for the future research.

ფარმაკოქიმია

Yucca gloriosa L. ფოთლების სტეროიდული გლიკოზიდები

ე. ქემერტელიძე*, მ. ბენიძე**, ა. სხირტლაძე**

* აკადემიკოსი, იველ ქუთათელაძის ფარმაკოქიმიის ინსტიტუტი, თბილისი

** იველ ქუთათელაძის ფარმაკოქიმიის ინსტიტუტი, თბილისი

საქართველოში ინტროდუცირებული მცენარის *Yucca gloriosa* L.-ის ჰაერმშრალ ფოთლებში 24 ფურო- და სპიროსტანოლური სტეროიდული გლიკოზიდის არსებობა იქნა დადგენილი. მათი უმეტესობა ტიგოგენინის წარმოებულა. გამოყოფილი და დახასიათებულია 14 გლიკოზიდი, მათ შორის 4 ახალი ორგანული ნივთიერება. სტრუქტურები მოწოდებულია კლასიკური და თანამედროვე სპექტრული ანალიზის მეთოდების გამოყენებით.

REFERENCES

1. E.P. Kemertelidze, T.A. Pkheidze (1972), Khim. Farm. Zhurnal, **6**: 44-47 (in Russian).
2. N.I. Menshova, N.N. Suvorov, E.P. Kemertelidze et al. (1974), Khim. Farm. Zhurnal (in Russian), **7**: 15-17.
3. N.Sh. Nadaraia, M.D. Mashkovskii, E.P. Kemertelidze et al. (1988), Khim. Farm. Zhurnal, **5**: 537-539 (in Russian).
4. A.M. Jorbenadze, A.Y. Stomberg (1972), Rastitelnye resursy, **1**: 97-103 (in Russian).
5. M.M. Benidze, T.A. Pkheidze, E.P. Kemertelidze (1991), Khim. Prirod. Soedin., **27**, 2: 295-296.
6. E.P. Kemertelidze, M.M. Benidze, A.V. Skhirtladze (2009), Khim. Farm. Zhurnal, **43**, 1: 27-29 (in Russian).
7. A. Skhirtladze, A. Plaza, M. Benidze, et al. (2006), Bioch. Syst. Ecol., **34**: 809-814.
8. P. Montoro, A. Skhirtladze, M. Benidze, et al. (2010), J. Pharm. Biom. Analysis, **52**: 791-795.
9. C. Bassarello, A. Skhirtladze, E. Kemertelidze, et al. (2007), Tetrahedron, **63**(1): 148-154.
10. P. Montoro, A. Skhirtladze, E. Kemertelidze et al. (2008), J. Pharm. Biom. Analysis, **47**: 854-859.
11. C. Bassarello, A. Skhirtladze, M. Benidze, E. Kemertelidze et al. (2007), J. Agr. Food Chem., **55**(16): 6636-6642.
12. P. Nigro, A. Skhirtladze et al. (2007), Life Sciences, **81**: 873-883.
13. A. Favel, E. Kemertelidze, M. Benidze, et al. (2005), Phytother. Res., **19**: 158 – 161.
14. E. Kemertelidze, M. Benidze (2001), Bull. Georg. Acad. Sci. **164**, 1: 91-93.
15. K.G. Gurielidze, Ts.A. Giorgadze, E.P. Kemertelidze, T.A. Pkheidze (1992), Fiziologiya rastenii, **39**, 2: 300-304 (in Russian).
16. M.M. Benidze, O.D. Dzhikiya, M.M. Vugalter, T.A. Pkheidze, E.P. Kemertelidze (1984), Khim. Prir. Soedin., **20**, 6: 744-747 (in Russian).

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